Technical abstract

Treatment is a critical problem in the management of severe Peripheral Artery Occlusive Disease (PAOD). Three therapeutic approaches are usually considered for patients suffering from PAOD (Isner & Rosenfield, 1993):

1) risk factor modification such as tobacco, dietary changes, etc..;
2) when possible, percutaneous transluminal angioplasty (PTA) to revascularize the ischemic limb;
3) bypass surgery with the use of prosthetic material or vein graft. Both PTA and especially surgery carry a significant morbidity/mortality in these patients who often suffer concomitantly from an already existing heart or disseminated vascular disease.

Therapeutic angiogenesis is a recent concept based on the use of angiogenic factors such as to promote neovascularization for the treatment of ischemic tissues. Both the administration of recombinant angiogenic factors, or gene transfer by viral or non viral DNA vectors that express these factors, were shown to induce angiogenesis in a number of preclinical animal models, particularly those with surgically-induced limb and coronary ischemia, as well as more recently in humans. Baumgartner et al. (1998) published results of a phase I clinical trial evaluating IM injection of a plasmid expressing VEGF-1 in 10 limbs of 9 patients with PAOD. This study established the proof of principle for therapeutic angiogenesis utilizing a non viral DNA vector. Results of this study indicated that IM injection of the plasmid DNA achieved expression of $VEGF_{165}$ transgene sufficient to induce clinical activity in patients with severe PAOD.

To deliver the angiogenic factor FGF-1 we engineered an expression cassette containing a naturally occurring truncated form of FGF-1 fused to a heterologous secretion signal (sp.FGF1) flanked by a strong viral promoter and polyadenylation sequences. This expression cassette was inserted into a proprietary plasmid backbone with a conditional origin of replication (pCOR). The resulting plasmid is referred to as NV1FGF.

Biological activity was demonstrated *in vitro.* Transient transfection of cells with plasmid followed by Northern blotting and Western blotting analysis demonstrated the functionality of *FGF-1* plasmid. Conditioned cell culture media were shown to stimulate proliferation of myoblast cells *in vitro,* confirming that the secreted sp.FGF1 exhibited biological mitogenic activity as expected.

Efficacy following IM *FGF-1* plasmid gene transfer into the ischemic muscle was demonstrated in a rabbit model of chronic hind limb ischemia. Escalating plasmid doses (100, 500 and 1000 µg) were administered 10 days after induction of limb ischemia and efficacy was assessed 30 days after treatment by measuring different anatomic and hemodynamic parameters. sp.FGF1 gene transfer led to the formation of angiographically visible collateral blood vessels. This increased angiogenesis resulted into improved blood supply to the limb as shown by improved limb blood pressure (ischemic/non ischemic) and intra-arterial blood flow in the ischemic limb at the highest dose (1000 µg) of sp.FGF1 plasmid in a dose dependent pattern.

Single IM administration of NV1FGF (1000 µg/kg) in rats led to expression of FGF-1 protein within the myofibers. The kinetic of *FGF-1* expression was delayed with reference to the DNA content: muscle fibers immunoreactive for *FGF-1* were not detectable at 4 hours post administration; they were rare at day 3 and more numerous at day 7. NV1FGF mRNA in the injected muscle was shown to highest at 7 days post administration, decrease by several orders of magnitude by 1 month and persist at this lower level for at least 4 months, The injection/expression of sp.FGF1 resulted in mild to moderate inflammation within the muscle which peaked at day 3 and decreased at day 7.

The biodistribution of NV1FGF after repeated administration included quantitative (Taqman PCR) evaluation of all major organs/tissues. At the levels tested (1 - 36 times the maximal clinical dose (80 - 2872 µg/kg)), the results obtained indicated that NV1FGF remains mostly restricted to the injection site, and only occasionally low amounts of plasmid vector may distribute to other organs and tissues. Absence of significant levels (above 10 copies/µg of DNA) of NV1FGF in the testes and ovaries suggests very low risk of transmission to germ cell line.